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Ultracomp™ MC1061/P3 *E. coli*

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Description

Transformation efficiency: 108 cfu/µg control DNA

Genotype of MC1061/P3: F- *hsd*R (rk-, mk+) *ara*D139 Δ(*ara*ABC-*leu*)7679 *gal*U *gal*K Δ*lac*X74 *rps*L *thi mcr*B {P3: Kan^R Amp^R (am) Tet^R (am)}

Contents

- 5 × 300 µL competent MC1061/P3 *E. coli*
- pUC19 plasmid DNA (10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8; 50 µL)
- SOC medium, 15 mL

Shipping and Storage

Ultracomp™ *E. coli* are shipped on dry ice. Upon receipt, store at −85°C to −68°C.

Transformation Procedure

Use the following protocol to transform MC1061/P3 competent E. coli.
Note: For MC1061/P3 transformed with a supF containing plasmid, we recommend performing double selection on 10 µg/mL tetracycline and 25 µg/mL ampicillin LB plates.

1. Thaw the tubes on ice. When the cell suspension is liquid, mix it by *gently* flicking the tubes. Immediately return the tubes to ice.
   IMPORTANT! *Keep the cells cold at all times.* Changes in temperature result in decreased transformation efficiency.
2. For each transformation, we recommend aliquoting 100 µL of cells into a pre-chilled 15 mL polypropylene tube (Falcon 2059 tubes are recommended). Immediately return the excess competent cells to −85°C to −68°C.
   Note: Each freeze-thaw cycle lowers the transformation efficiency.
3. Add the DNA solution in a volume of 1–5 µL per 100 µL cells and swirl the tube to evenly mix the DNA and cells. Incubate the solution on ice for 30 minutes. **Note:** For control DNA, use 1 µL (10 pg).
4. Heat-shock the cells by placing the tube in a 42°C water bath for 30 seconds. Remove and chill the cells on ice for 2 minutes.
5. Add 900 µL of SOC medium to the cells.
6. Incubate the tube at 37°C with moderate agitation (225 rpm) for 60 minutes.

Plate 100 µL on LB plates containing the appropriate selective agent. Cells can be concentrated by centrifugation at 400 × g (maximum) for 5 minutes and resuspended in a small volume of SOC. For the control DNA, plate 25 µL and 100 µL of cells on LB agar with 100 µg/mL of ampicillin.

Growth of *E. coli* Carrying the P3 Plasmid

E. coli cells carrying the P3 plasmid are designed for transformation of vectors that encode the tyrosine tRNA suppressor (synthetic *sup*F gene).

P3 Plasmid

The P3 plasmid is a low-copy-number, 60 kb plasmid that carries the drug resistance markers for kanamycin, tetracycline, and ampicillin. The fully active kanamycin gene is used to select for cells carrying P3. The tetracycline and ampicillin genes carry amber mutations that render the genes inactive during normal growth and replication of the bacteria. Upon transformation of a vector carrying the suppressor F gene (such as pcDNA1.1 or pCDM8), the amber mutations in the tetracycline and ampicillin genes on the P3 plasmid are suppressed, and the *E. coli* are resistant to these antibiotics.

Prepare the *E. coli* Carrying the P3 Plasmid

To prepare competent cells containing P3 for transformation with a SupF plasmid, it is important to start a culture from a non-revertant colony (sensitive to both ampicillin and tetracycline). Use the following instructions:

1. Remove a small amount of bacteria from a stab or a glycerol stock and resuspend it in 250 µL of liquid media such as LB.
2. Streak bacteria from this suspension onto agar plates containing 40 µg/mL kanamycin for selection of *E. coli* containing P3.
3. Incubate plates overnight at 37°C.
4. Patch 20–30 single, KanR colonies in a grid pattern on separate tetracycline (10 µg/mL), ampicillin (50 µg/mL), and fresh kanamycin plates.
5. Incubate the plates overnight at 37°C, then select the colonies from the kanamycin plate that exhibit sensitivity to both tetracycline and ampicillin. Use these colonies to prepare the competent cells.

Reversion Rates

The spontaneous reversion rate for the amber mutation in the ampicillin marker is 5%, while the reversion rate for the amber mutation in the tetracycline marker is 1%.

Minimizing the Reversion Rates

Because revertant cells (without the SupF plasmid) grow much faster than plasmids with the SupF plasmid, reversion rates must be minimized using both tetracycline (10 µg/mL) and ampicillin (50 µg/mL) together for selection of *E. coli* (P3) cells transformed with the SupF plasmid.

Information for European Customers

The MC1061/P3 *E. coli* strain is genetically modified and carries the P3 plasmid containing the kanamycin, tetracycline, and ampicillin resistance genes. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Safety Data Sheets (SDSs)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

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